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I arrived in Joshua Lederberg's laboratory in Madison, Wisconsin, in early July of 1948. The laboratory was a 20-by-30-foot room in the basement of the genetics building, which looked like an overgrown log cabin. At the time, little was known about the nature of the gene, although the one gene:one enzyme hypothesis of George Beadle and Edward Tatum (Beadle and Tatum, 1941) gave some insight into function. The process of transformation, whereby a bacterium incorporates DNA from the media into its genome, had been discovered in Pneumococcus in 1944 (Avery, Macleod, and McCarty, 1944) and was still considered questionable when I began working. This experiment showed that DNA was the genetic material. In 1946, Lederberg had discovered that E. coli bacteria could exchange genetic material between the cells by the process of conjugation (Lederberg, 1947). However, in the 1940s, most scientists thought bacteria to be asexual and that it could only occasionally undergo morphogenetic changes to filaments or endospores. They did not believe in these new developments and in fact, they didn't believe bacteria had genes but rather that they inherited acquired characteristics in the manner described by Lamarck. My task as graduate student was to determine whether conjugation occurs only in E. coli or was also found in other types of bacteria. I began by studying the closely related bacterium Salmonella, which causes typhoid fever. The different species of Salmonella are classified on the basis of serotypes of their surface antigens and their flagellar antigens. The sets of phenotypes looked as if they might have been constructed by genetic recombination because they seemed to be combinations of each other.

Figure 1 When two different mutants are plated together on minimal medium, growth only occurs if recombination generates a strain that has wild-type alleles at both loci. Download

The method Lederberg used to demonstrate genetic recombination resulting from conjugation was to cross two strains that contained complementary nutritional requirements and to select for bacteria that had lost the requirements. For example, one strain might lack the ability to grow on media that doesn't have leucine, while the other can grow on such media but can't grow on media without phenylalanine. A bacterial cross was accomplished by spreading one tenth of a milliliter of each parent together or separately on the surface of a minimal medium agar plate, which lacks all of the nutrients a wild type bacterium can synthesize. Figure 1 shows that bacterial colonies should be seen only when recombination has taken place. Generally strains with more than one nutritional requirement were used to prevent spontaneous reversion back to wild type. Reversion occurs at low frequency in a growing population and is caused by a change in DNA that either reverses the original alteration or compensates for it.

Figure 2 Wild-type bacteria die in penicillin, but bacteria survive if they cannot grow because they have a mutation that creates a requirement for some nutrient. Download

In order to carry out my studies on conjugation in Salmonella it was necessary for me to isolate nutritional mutants in the strains I wanted to use. At the time, isolation of such mutants was exceedingly difficult. However, within four weeks of my arrival Lederberg suggested a simple means for the isolation of nutritional mutants. The isolation was based on the fact that penicillin only kills growing cells because it inhibits the cell wall synthesis required to accommodate a growing cell. Thus if mutagenized bacteria were placed in a minimal medium, mutants that could not synthesize a particular nutrient would not grow and hence would not be killed by penicillin. The bacteria could then be plated on media that contain the necessary nutrients, and growth would resume. Figure 2 shows how penicillin selection is used to enrich for nutritional mutants. Within a few days I was able to show that this technique was effective and probably isolated more mutants than I could analyze in a lifetime (Lederberg and Zinder, 1948). My starting material was 22 strains of the bacterial species S. typhimurium that we received from Sweden in the diplomatic pouch. The strains had all been isolated from cases of Salmonella gastroenteritis and were classified into 22 strain types on the basis of their sensitivity to a series of bacterial viruses. What with working toward a master's degree, making the media, and cleaning glassware for the laboratory and helping Joshua with experiments, I spent the next year-and-a-half making complementary pairs of mutants needed for the attempt at crosses. In addition I took the phage course. Finally, I had the collection of mutants I needed and began to cross them to each other. As time passed and more of the potential crosses were tried, certain things became apparent. There was no evidence of genetic exchange with any of the intrastrain crosses. There were a few interstrain crosses that seemed to work and in each case the presence of a particular parental strain was critical. The most productive cross was between two strains labeled LT2 and LT22. LT2 had mutations that caused it to require the amino acids methionine and histidine. LT22 had two mutations, the first of which caused it to require the amino acids phenylalanine and tyrosine and the second added a requirement for the related aromatic amino acid tryptophan. These were selective markers; when plated on minimal media, no bacterial colonies would form unless the nonmutant state of these genes was present. We found that we could get recombination and therefore growth on minimal media when these two strains were crossed. There are other genetic markers that can allow growth in either mutant or nonmutant form and thus are not necessarily selective. For example, if Salmonella cannot ferment a particular sugar it will use amino acids as an energy source; thus the ability to ferment the sugar is not absolutely required for growth. Fermentation mutants can be distinguished from wild-type bacteria by adding an indicator dye to the medium; the nonfermenters are pink and the fermenters are purple.

Figure 3 The transfer of selective and nonselective markers can be tested for in a single assay. Download

We examined the transfer of nonselective markers in Salmonella as a more rigorous test of genetic exchange, since there is no pressure to acquire the new traits. We crossed two strains that had complementary nutritional requirements (inability to synthesize a particular amino acid) as well as complementary fermentation requirements (inability to utilize a particular sugar as a carbon source). When plated on minimal media supplemented with a particular sugar source, only strains that have gained the ability to make all the essential amino acids will grow. Figure 3 shows that if a strain has also picked up the ability to ferment the sugar, then the bacterial colony will be purple. We found that the nonselective markers were not

transferred, as indicated by the lack of purple colonies. Further investigation revealed an interesting difference in the results obtained with Salmonella compared with those obtained previously with E. coli. With the crosses in E. coli all pairs of nonselective markers would be seen in the recombinant progeny. This meant that both parents could contribute genes to the progeny. However, with the Salmonella crosses in which an unselected marker from either the LT22 or the LT2 parent could be identified in the resulting colonies (not shown here), only the unselected markers from the LT22 parent were seen.

The experiment

Figure 4 Different strains of bacteria were grown on either side of a filter. Recombinant bacteria appeared on one side of the filter, implying that material smaller than the bacteria must have passed through the filter from the other side. Download

We asked whether the parental bacteria had to come in contact with each other for the reaction to occur as had been shown for E. coli conjugation. We used a U-shaped glass tube that had a bacterial sterile filter in its middle (Davis, 1950). Each arm was inoculated with a different parent in growth medium and the liquid was flushed back and forth as the cultures grew. Figure 4 shows that recombinant bacteria were found only in the LT22 side of the tube. We concluded that something small could pass through the filter to produce the effect. Supernatants from our individual cultures alone did nothing, but supernatants from mixed cultures could produce recombinants of LT22. We called the activity that transferred the genetic markers FA (filterable activity). We had demonstrated that FA did not transfer nonselective markers. However, given the right circumstances a nonselective marker can be made selective. For example, a xylose fermentation mutant can grow on a plate supplemented with all of the essential amino acids. But after a while, those nutrients will be used up and only bacteria that can ferment xylose will continue to grow; thus xylose-fermentation becomes a selectable trait.

Figure 5 When a filtrate (FA) is prepared from a strain that had no nutritional mutations (prototroph) and incubated with a nutritional mutant, the nutritional mutant gains the ability to grow on medium lacking any one of several growth factors. In each case the bacteria have only gained the single genetic marker for which one has selected. The genetic activity of transduction correlates with the properties of the bacteriophage. Download

We tested whether FA could transfer the markers under these conditions. We isolated FA from the supernatant of a bacterial strain that had no nutritional defects and was able to utilize galactose and xylose as carbon sources. This was accomplished by incubating the strain with a lysogenic phage which grew on the strain and was released by its lysis. We incubated the FA with a bacterial strain that required phenylalanine, tyrosine, and tryptophan in the media and was also unable to ferment galactose and xylose. Then we plated the bacteria on three different types of plates: minimal media, complete media supplemented with xylose, and complete media supplemented with galactose. Figure 5 shows that in each case, only the selected marker is found in the recombinant progeny. Thus the phenomenon resembled DNA-mediated transformation in Pneumococcus, in that only one genetic marker at a time would be changed.

Figure 6 Transduction works by replacing all or part of the phage genome with bacterial DNA. Download

With one more fact, all of the above could be explained. Even though, at the time, there was disagreement as to whether bacteria could carry viruses, we had found many of them in our cultures. We reasoned that LT22 released a phage that grew on LT2 and produced the genetic material that returned through a filter. With further experiments it was possible to show that the active particle was the phage itself. Figure 6 shows how bacterial DNA carried in a phage particle could give the results we saw in the U-tube experiment. How was the phage eliciting specific changes in the genetic constitution of the bacteria it infected? We now know that bacteria can be infected by phage in two different ways: lysogenically and lytically. In the lytic pathway, phage inject their DNA into the bacterium, take over the cell's replication machinery, produce many new phage and rapidly cause lysis of the bacterium, releasing the newly generated phage into the media. In the lysogenic pathway, the phage DNA is incorporated into the bacterial host's genome. The phage suppresses the lytic pathway and reproduces along with the host until conditions become adverse for bacterial growth. The phage senses this and excises its DNA from the bacterial genome, begins the lytic pathway, and the cell soon lyses.

Figure 7 The right panel shows how a transducing particle is formed by an error in packaging during the lytic cycle of a phage. The left panel shows how the incorrectly packaged bacterial DNA can become incorporated in the newly infected bacterium's genome. Download

It turned out, in the case of the phage growing on LT2, that when the phage was induced to excise from the genome and repackage its DNA into phage particles, the entire phage genome was replaced by an equivalent amount of bacterial genome (Ikeda and Tomizawa, 1965). The phage carried this bacterial DNA back to the LT22 strain and injected it into the bacterium, whereby it recombined, producing the effects we had seen. The phenomenon was called General Transduction (to lead across) in that all bacterial genes could be carried by the phage and recombine with the newly infected bacterium's genome (Zinder and Lederberg, 1952). However, the phage can only package a small amount of genetic material, thus only a few genes are carried at one time. Figure 7 illustrates the process of transducing particle formation and function. Shortly thereafter, specialized transduction was discovered using the bacteriophage lambda (Morse, Lederberg, and Lederberg, 1956). The temperate bacteriophages would insert themselves into the bacterial genome at a specific site. When they excised themselves, they would occasionally make a mistake and a proximal segment of the bacterial genome will replace some of the phage's genes. Upon infection of a new host these bacterial genes would replace their homologues. For the phage lambda, it was the genes affecting galactose fermentation that were involved. Later, when its position on the chromosome was found, PLT22 would transduce the genes for proline metabolism. Thus while lambda could only cause specific transduction, PLT22 could do both specific and general.

The legacy It was the existence of transduction that led to the idea of recombinant DNA; after all, both involve the fusing together of two different pieces of DNA, generally one of which can replicate (Jackson, Symons, and Berg, 1972). The goal of recombinant DNA was to be able to do with eukaryotic cells the kinds of genetics that transduction allowed with bacteria. In addition, it became a way to move genes that made important proteins to organisms in

which they might produce large amounts of the important protein. And of course it was recombinant DNA which opened the way to sequencing of DNA from any organism. Production of tumors by retroviruses is also an example of transduction. These retroviruses insert themselves into a cell's genome and again by error exchange part of the genome for mutant cell genes (Stehelin et al., 1976). The genes picked up are important cellular genes involved in regulation of the cell cycle and gene regulation. When mutant, they are the dominant cancer-causing genes, and are known by their acronyms such as src, abl, and ras. Given that transduction is a sexual mechanism, it would be surprising if it didn't play an important role in evolution. Many different organisms have had their DNA sequences completed, especially microbes. It is now clear that there has been a great deal of horizontal gene transfer in evolution. The major vehicle for such transfers was probably transduction as sequences of bacteria contain a large number of pieces of phage genes. When genes can move, even partial genomes, it has profound effects on living organisms.

The Author

Norton D. Zinder, John D. Rockefeller, Jr. Professor of The Rockefeller University and cohead of a laboratory of genetics was the first chairman (1989-1993) of the Program Advisory Committee on the Human Genome convened by the National Institutes of Health. Dr. Zinder's studies of the genetics of bacteria and the properties of bacteriophages, viruses that infect bacteria, have provided important information on the mechanisms of heredity. In early experiments conducted in collaboration with Dr. Joshua Lederberg, former president of The Rockefeller University, Dr. Zinder discovered that bacteriophages can carry genetic material from donor to recipient bacteria. This process, called transduction, is one of nature's models for recombinant DNA technology and viral oncology and has also been widely applied in the development of research methods for the study of bacterial genetics. With colleagues at Rockefeller, Dr. Zinder later discovered the first bacteriophages known to contain RNA. He also demonstrated that RNA phage replication is not dependent on DNA, a finding which provided further evidence that RNA carries the blueprint for the manufacture of protein. The first protein synthesized outside the cell was made in his laboratory, providing clues as to the start and stop signals of the genetic code. Work on the filamentous bacteriophage (F1) has led to the ability to convert any piece of DNA to a single strand and thereby being able to sequence it. Born in New York, Dr. Zinder received an A.B. degree from Columbia University in 1947 and a Ph.D. from the University of Wisconsin in 1952, the same year he joined The Rockefeller University. He was appointed professor in 1964 and John D. Rockefeller, Jr. Professor in 1977. A member of the National Academy of Sciences, Dr. Zinder has served on many scientific committees and received numerous awards, including the Scientific Freedom and Responsibility Award given by the American Association for the Advancement of Science.

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